Introduction to Monitoring for Monarch Conservation

Monarch butterflies (*Danaus plexippus*) are an iconic species with an annual, multigenerational, migratory life cycle and a cross-continental migratory range covering portions of Canada, the US and Mexico. To complete their life cycle, monarch caterpillars must feed on milkweed (*Asclepias* spp.) plants while adults feed on nectar from a variety of blooming plants. Threatened by habitat loss, climate change, pesticide applications, natural enemies and other abiotic and biotic stressors, however, monarch butterfly populations are in decline. Though monarch conservation is a trinational concern, spatial and temporal distribution of monarchs and their habitat is poorly understood in the US.

The Monarch Conservation Science Partnership (MCSP) is a consortium of scientists and conservation professionals affiliated with government agencies, academic institutions or non-government organizations. A subgroup of the science partnership formed a Monitoring Team to desing a spatially balanced sampling scheme (GRTS, Generalized Random Tessellation Stratified draw) and a National Protocol Framework, modeled after recent US Fish and Wildlife Service standards, to monitor temporal and spatial distribution of monarchs and their habitat across the US landscape (Thogmartin et al. 2015 unpublished report). Standard Operating Procedures (or SOPs) in this document provide the details for data collection and management activities and are part of the monitoring protocol framework These procedures will be tested during a Monitoring Trial (May –October 2016) at three National Wildlife Refuges (Neal Smith NWR, Waubay NWR, Washita NWR) and surrounding private lands.

Following the design and purpose of monitoring data to support several models that have been used to evaluate demographic trends in the population of the monarch that migrates to Mexico and conservation targets, data will be collected at each refuge and surrounding lands in the following strata: unprotected and protected grasslands, roadsides, cultivated agricultural fields, and lands enrolled in the Conservation Reserve Program (CRP). The Chicago Field Museum will collaboratively test this protocol in urban/suburban settings (2016) but will employ a different methodology to select sample sites. Some of the sampling around refuges will entail gaining access to privately owned lands. The Natural Resources Conservation Service will be helping the monitoring staff with securing that access.

At selected sites, six SOPs will be used to monitor for monarchs and their habitat. These SOPs instruct users on how to: a) establish sites (SOP 1), b) ground truth sites (SOP 1), c) conduct a rapid assessment of monarch habitat to determine if more in-depth monitoring is warranted (SOP 1), d) monitor sites (indicated by SOP 1) by measuring select attributes of monarchs and their habitat (SOPs 2, 3 and 4) and e) enter and manage data (SOP 5). SOP 6, which involves estimating Red Imported Fire Ant density and biomass, will be tested separately by Dr. Kristen Baum in 2016.

Authors—The methodology being tested this year is a product of past monitoring or study methods used in both academic and citizen science (Oberhauser et al. 2009). The on site sampling design and modification of existing protocol materials were developed by the following people:

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Expected Results—Implementation of SOPs at priority draw locations during this 2016 Monitoring Trial will test methodology and logistics while also providing a means to estimate sampling variability across a broad range of conditions in each sampling stratum. Sampling and measuring methods likely greater that may be required but are purposely designed to allow examination of thresholds in information loss with reduced effort. The time required for implementation of each SOP will allow estimates of cost for each SOP on a per sample unit.

Future Directions—Following the Monitoring Trial, the GRTS draw will be expanded to either a national or trinational level. SOPs will be modified based on trial results and compiled into a USFWS National Protocol Framework which will be made publically available. In 2017, the MCSP will seek broad-scale (national or trinational) implementation of the National Protocol Framework through citizen scientists and other monitoring entities at priority GRTS sites across strata. Results from broad-scale implementation of the framework will help the MCSP to track spatial distribution and changes in the U.S. monarch population and related habitat resources over time and possibly provide reference sites for companion monitoring proposed for quantifying the contribution of local projects to increases in milkweed, nectar plants and butterfly habitat use and productivity.

Monarch Butterfly Biology and Conservation

Monarch Butterflies are a Migratory Species—Monarch butterflies (*Danaus plexippus*) are an iconic species native to Central and North America with an annual, multigenerational, migratory life cycle (Appendix A). Each monarch generation begins with eggs laid on milkweed (*Asclepias* spp.) which hatch after ~3-8 days (depending on temperature). Caterpillars consume milkweed, completing 5 growth stages called instars. This process takes ~ 9-14 days. Mature 5th instar caterpillars will seek a sheltered location to pupate where they form a chrysalis and undergo complete metamorphosis. After ~8-15 days, adult butterflies emerge.

Each fall, millions of monarch butterflies east of the Rocky Mountains migrate from as far north as Canada to the central Mexican State of Michoacán. There, butterflies overwinter in dense clusters, blanketing oyamel fir trees in mountainous forest preserves. In the spring, clusters disperse and monarch butterflies mate and migrate northward into the southern US. This first

wave of monarchs lays eggs on milkweed plants (*Asclepias* spp.) and after completing its development from caterpillar to adult, the next generation continues the journey north, seeking milkweed. This northward, generational voyage continues until a fourth generation emerges as far north as southern Canada in the late summer. Shortening days and other cues indicate that the summer is coming to an end. Rather than mating, this fourth generation enters a state of reproductive diapause and migrates south all the way to central Mexico. Along the ~2500 mile trip, migrating monarchs feed on nectar to build fat reserves that will sustain them during the winter months in Mexico. West of the Rocky Mountains, monarch butterflies overwinter in groves along the Pacific coast in California. In the spring, monarchs disperse and subsequent generations populate regions to the north, east and south.

Monarch Butterflies are in Decline—Annual measurements of total area occupied by overwintering monarchs in Mexico indicate that the Eastern migratory population has generally declined since 1996, with the lowest recorded areas in the winters of 2012-2015 (see Figure 1). Using predictive modeling, researchers found that the Eastern monarch population is at significant risk of quasi-extinction (dwindling to such a small size that the migratory phenomenon collapses and recovery becomes impossible), unless conservation action is taken to augment monarch populations size (Semmens et al., 2016).

Monarch Butterflies are Threatened by Several Stressors—Monarch populations are thought to be in decline for a number of reasons including: 1) breeding habitat loss, 2) overwintering habitat loss, 3) climate change, 4) pesticide exposure and 5) natural enemies (see Appendix A).

1. *Breeding habitat loss:* Immature monarch butterflies (caterpillars) only eat milkweed (*Asclepias* spp). Thus, in order to reproduce, adult female monarchs must locate and lay eggs on milkweed plants. Intensive agricultural practices including spraying the herbicide glyphosate, have dramatically reduced milkweed populations in the Midwestern US (Corn Belt Region). Prior to widespread adoption of herbicide-resistant crops, the Corn Belt was an important source of milkweed habitat for monarchs. Beyond agricultural fields, urban sprawl and other practices such as mowing roadsides remove monarch habitat from the landscape.
   In addition to milkweed availability limiting reproduction during spring and summer months, restricted availability of quality nectar plants may also hinder monarch population growth. Adult monarchs feed on nectar from a number of plants (not just milkweed) and access to nectar resources may be especially important for fall migratory monarchs which must feed on nectar to build fat stores in preparation for overwintering in Mexico or California. Thus, general degradation of pollinator habitat across the US landscape may be contributing to monarch population declines.

2. *Overwintering habitat loss:* Overwintering habitat destruction in both Mexico and California also threaten monarch populations. Monarchs require specific, sheltered microclimates (temperature, humidity) afforded by oyamel fir forests (Mexico) and tree groves (California) in order to successfully overwinter. Legal and illegal logging in Mexican butterfly preserves in addition to development in California and encroachment of non-native tree species threaten the integrity and continued availability of overwintering grounds

3. *Climate change:* It is difficult to predict exactly how climate change will affect monarchs. Changes in climate may affect the distribution and timing of availability of milkweed and nectar plants and contribute to further degradation of overwintering grounds in California and Mexico. As a migratory species, monarchs are able to travel long distances to find suitable habitat. However, whether suitable breeding and overwintering habitat will continue to be available, and whether monarchs will be able to shift their range to exploit these resources remains to be seen.

4. *Pesticide exposure:* Milkweed and monarchs are exposed to different insecticides across landscapes. In agricultural and urban/suburban settings, insecticides may be applied to control herbivorous pest insects. Furthermore, pesticides are applied to bodies of water and as fog sprays to control larval and adult mosquitoes. While monarchs and other beneficial insects are not direct targets of these applications, exposure to these chemicals may result in sublethal or lethal effects.

5. *Natural enemies*: Though monarch caterpillars sequester toxins from milkweed plants which confer protection to both caterpillars and adults, a number of arthropods (ants, lacewing larvae, spiders, beetles, Chinese preying mantids) and some bird species prey upon monarch caterpillars or adults. Furthermore, there are parasitoid flies and wasps and that lay their eggs inside caterpillars and monarch chrysalises respectively. These larval flies and wasps develop inside the parasitized monarch and when the monarch pupates (forms a chrysalis), parasitoid larvae kill the monarch and use the monarch butterfly host resources to complete their own development. Finally, monarchs suffer from a number of viral, fungal, bacterial and protozoan diseases. The best-studied monarch disease is caused by the protozoan parasite *Ophryocystis elektroschirra* (OE).

Broad-scale Surveillance Monitoring for Conservation Models

Study Rationale—Monitoring monarch butterflies and their habitat is challenging, but necessary for conservation. To conserve monarchs we need a better understanding of how monarch habitat is distributed throughout the US landscape and regionally where the different life stages of monarchs are supported (Appendixes A and B). Because of their multigenerational, migratory life cycle and continental range, tracking monarch populations and their habitat poses a large challenge. Several entities, including citizen science programs, NGOs, federal and state agencies and academic institutions have a rich history of monitoring monarch habitat and/or monarch populations. However, no one entity comprehensively monitors all monarch life stages and the abundance and distribution of their habitat using a spatially balanced sampling scheme on a continental scale.

Monitoring Site Selection—The MCSP will use a GRTS (Generalized Random Tessellation Stratified) draw to select monitoring sites. The MCSP will use a GRTS (Generalized Random Tessellation Stratified) draw to select spatially balanced, priority sites for monitoring. Under a GRTS sampling scheme, a geographic area to be sampled is stratified by land cover type. Since monarchs may reproduce on milkweed located in cities, to milkweed on roadsides, to milkweed found in open fields, the MCSP is stratifying US land cover into the following land cover categories of interest: 1) unprotected grasslands, 2) protected grasslands, 3) ROW (Right of Way) habitats (e.g. roadsides, powerline corridors, etc.), 4) cultivated agricultural lands, 5) CRP

(Conservation Reserve Program) lands and 6) urban/suburban spaces. Applying a GRTS draw to stratified US land cover data produces a list of priority sites for sampling within each stratum of interest. Locations of sites within a given stratum are spatially balanced: sites are distributed evenly so that sites are not clustered too close or spread far apart from each other as might happen by chance using a purely random design. Depending on a site's priority ranking given in the GRTS list, data collected from that site will be weighed more (or less) heavily in a final analysis. A strength of using a GRTS design is that data can be collected from virtually anywhere as long as the site fits into one of the strata of interest. The catch is that most sites will not be priority sites for monitoring and data contributed by monitoring low-priority sites will not weigh heavily in an analysis. Thus, the GRTS design will allow citizen scientists, academic institutions, federal agencies, NGOs and other entities conducting monitoring to submit data from any sites provided that: 1) sites fit into a stratum of interest and 2) this protocol or compatible protocols are used to collect data so that data can be compared across studies. However, the MCSP will encourage monitoring entities to monitor priority sites within each stratum to strengthen subsequent analyses.

For this Monitoring Trial (2016), sites on refuges and surrounding private lands have been chosen through three separate 'mini-GRTS' draws, with one draw per refuge and associated lands at Neal Smith NWR (National Wildlife Refuge), Waubay NWR and Washita NWR respectively. \ USFWS and contracted biological technicians (biotechs) will monitor sites in the following strata: 1) unprotected grasslands, 2) protected grasslands, 3) ROW habitats (roadsides only), 4) cultivated agricultural lands and 5) CRP lands. As part of a separate but collaborative monitoring effort, the Chicago Field Museum has hired biological technicians to test this monitoring protocol in the urban/suburban stratum in Minneapolis-St. Paul (MN). While sampling procedures will be shared across studies, sites will be chosen for this urban/suburban stratum using criteria selected by the Chicago Field Museum.

In 2017, the MCSP is considering expanding the GRTS draw to either the national (48 contiguous US states) or trinational (Canada, US, Mexico) level and identify priority sites for monitoring within the following strata: 1) unprotected grasslands, 2) protected grasslands, 3) ROW (Right of Way) habitats (e.g. roadsides, powerline corridors, etc.), 4) cultivated agricultural lands, 5) CRP (Conservation Reserve Program) lands and 6) urban/suburban spaces.

Testing Operating Procedures—During the 2016 monitoring trial, USFWS biotechs at each refuge will visit priority sites in each stratum identified by GRTS draw (see previous section) and establish plots by flagging GPS-designated points (SOP 1). Notably, plot dimensions and associated sampling instructions differ across some strata types (ROW habitats along roadsides are long and narrow, while grasslands, CRP and agricultural fields are generally more square. Thus ROW plot dimensions and sampling instructions differ from those employed in grasslands, agricultural fields or CRP fields). Once plot corners are established, biotechs will ground truth the site (SOP 1). For example, if a site is supposed to be a protected grassland, but upon visiting the site, the biotech discovers that a significant portion of the site is forested, the site will be rejected for inclusion in the study.

Once a site has been ground truthed, biotechs will conduct a rapid assessment of monarch habitat (SOP 1). Biotechs will walk a predefined trajectory through the plot, noting milkweed plants and plant community characteristics (SOP 1). If enough monarch habitat is present to warrant in-depth monitoring, biotechs will return to the site to measure: a) relative abundance of blooming

nectar plants (SOP 2), milkweed density (SOP 2) and monarch egg and larval density per milkweed plant (SOP 2). While monitoring plants and immature monarch stages, biotechs may collect late instar caterpillars, rear them to adulthood and evaluate specimens for protozoan parasites or tachinid fly parasitoids (SOP 3). During site visits, biotechs will also count adult butterflies and record their behaviors while walking a predefined trajectory through plots (SOP 4). Sites that require in-depth monitoring (SOPs 2-4) will be revisited at least every three weeks, and when appropriate, every 2 weeks (SOP 4 only). Sites that do not require in-depth monitoring will not need to be revisited. Biotechs will enter and manage data according to instructions provided in SOP 5.

Feedback on SOP clarity provided by biotechs and documented effort will guide troubleshooting and modifications to SOPs during the 2016 Monitoring Trial. After the trial is complete, collected data will be analyzed by the MCSP and partners to determine the level of effort needed for each SOP (e.g. What is the minimum number of subplots needed in order to accurately capture variation for a given monitoring attribute within a plot in SOP 2?). Based on these analyses, SOPs will be modified and compiled into a draft USFWS National Protocol Framework. During this time, the MCSP may also develop simplified versions of some SOPs to make them accessible to different monitoring audiences. The draft USFWS National Protocol Framework, incorporating all SOP versions, will be made available for internal and external peer review in late 2016 or early 2017. After comments and suggestions from the peer review process are integrated, the National Protocol Framework will be made publically available for any monitoring entity to use during the 2017 field season.

While data are analyzed and SOPs are modified during the fall (2016) – spring (2017), the MCSP Monitoring Team will: 1) conduct a formal large scale GRTS draw (see above), 2) develop a database to house submission of data in 2017, and 3) develop a website that will allow different monitoring entities to access information about priority GRTS sites, download the National Protocol Framework SOPs, and upload collected data. Meanwhile, the MCSP Engagement Team will work to increase awareness and collaboration in implementing National Protocol Framework and promote monitoring at GRTS priority sites by different monitoring entities (e.g. citizen scientists, federal and state programs, NGOs, academic institutions, etc.).

This process is intended to be both integrative and iterative. Rather than developing new SOPs, wherever possible, the MCSP has integrated existing SOPs from other monitoring programs to facilitate future coordination across monitoring entities. For example, instructions for milkweed, monarch egg and monarch larvae monitoring (SOP 2) and instructions for rearing caterpillars and sampling for parasites and parasitoids (SOP 3) are adapted from the MLMP Citizen Science Program (Monarch Larva Monitoring Project, 2016). Furthermore, by using the GRTS sampling scheme, this framework will be able to integrate information from monitoring entities that contribute data from any appropriate site (though high priority GRTS sites will be weighed the heaviest in the final analysis, see above). By collaborating with other monitoring entities, the MCSP will seek to "crosswalk" across data sets, to identify data gaps and to circle back and address those gaps. This iterative process will provide information about the distribution of monarchs and their habitat through time and space and inform conservation efforts.

References

Oberhauser K, Batalden R, Howard E. 2009. Monarch Butterfly Monitoring in North America: Overview of Initiatives and Protocols. Commission for Environment, Montreal (Quebec), Canada.

Thogmartin WE, Ward JP, Grundel R, Oberhauser K, Newman J, Weltzin J. July 2015 Draft. Integrated Strategy for Understanding Effectiveness of Monarch Conservation. Unpublished Working Paper, USFWS, Natural Resources Program Center, Fort Collins, CO.

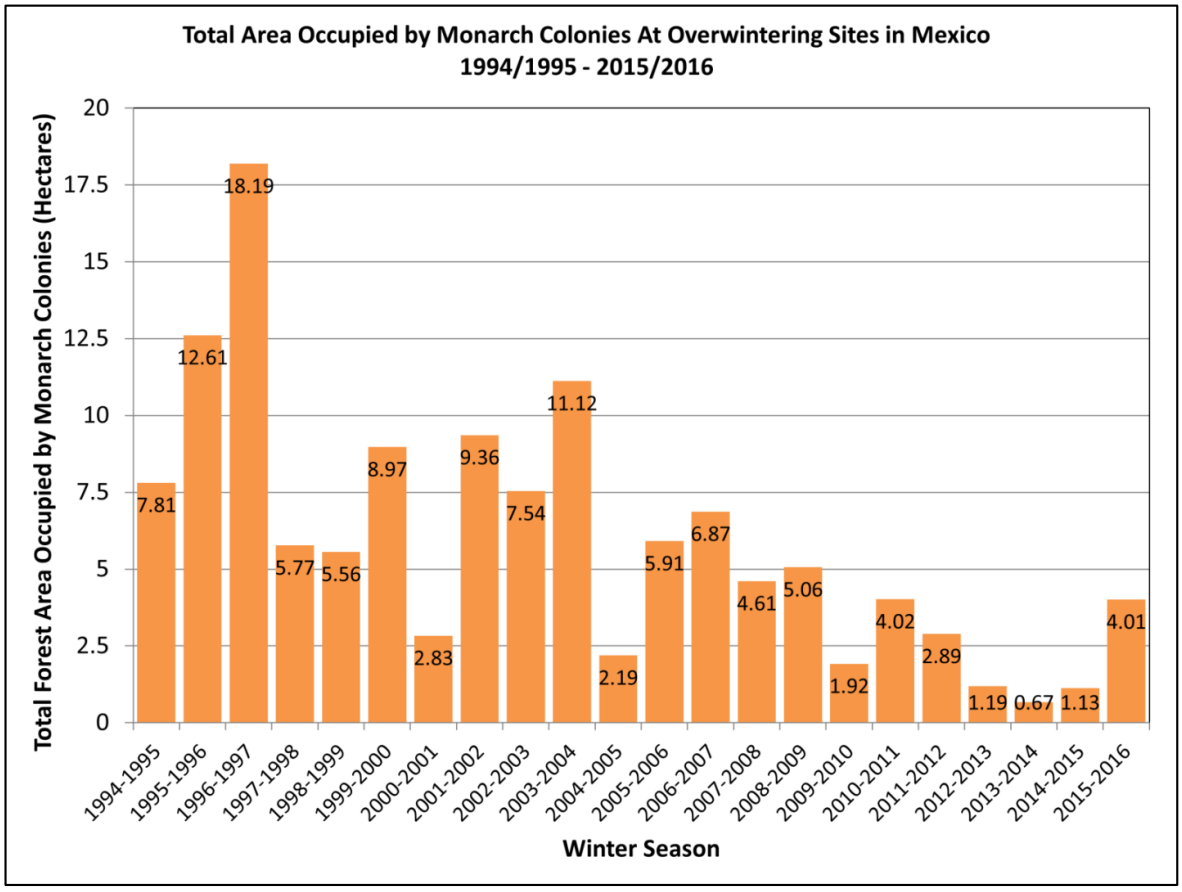

Figure 1. Annual, total area occupied by monarch butterfly colonies at overwintering sites in Mexico. Best estimates suggest that each hectare occupied contains ~37.5 million monarchs. Data from 1994-2003 were collected by the Monarch Butterfly Biosphere Reserve (MBBR) of the National Commission of Protected Natural Areas (CONANP) in Mexico. Data collected from 2004-2016 were collected by the WWF-Telcel Alliance, in coordination with the Directorate of the MBBR (Monarch Joint Venture, 2016b).